## COLOR CODING KEY

**Characterization**
**Construct**
**Data Collection/Analysis**
**Improvement of Biobrick**
**Plant-Care**
**Plasmid**
**Plasmid/Construct Design**
**Preparatory Work**

---

**Sunday July 14th**

- Created and autoclaved LB media
- Streaking:
  - Streaked plates with 500uL of AMP and 500mL LB broth
  - 100uL of Hifi/Positive control transformed cells
- Transformation:
  - Used HiFi kit
- Plants:
  - All inoculated leaves have similar numbers of inoculation sites visible
  - Old inoculated plants had stunted growth
    - Sheared off big leaves for visualization of stem and other leaves

---

**Monday July 15th**

- Liquid inoculation of plates from the previous day
- Plants:
  - TMV has not entered the major leaf vasculature yet

---

**Tuesday July 16th**

- Liquid cultures taken out
  - Cultures appeared clear
  - Cultures did not pellet when centrifuged
- Plates:
  - Made CAM plates

    - Prepare the CAM from the powder in the -20C fridge
- Plants:
    - Transplanted 2-week sprouts into 16 square wells and circular ports + osmocote
    - Thinned the plants and sowed another batch
    - Sub Irrigated with fertilizer

---

**Wednesday July 17th**

- Liquid inoculation:
    - 3 tubes of HFA
    - 3 tubes of HFB
    - 1 tube for positive control
    - 1 tube for positive control A
    - 1 tube for positive control B
    - 1 tube for negative control
- Created YPD agar plates
    - Used 13g of YPD agar powder in 200mL MilliQ water
    - Poured 6 plates of YPD agar
- Streaking:
    - 1 control plate
    - 2 of Rowley's XRN1 knockout plate
    - 2 XRN1 knockout plate
    - Plates were placed into the incubator at 35C
- Plants:
    - Created our own inoculation solution
    - Gained five 2 mL tubes of freeze dried ground up leaves

---

**Thursday July 18th**

- Liquid inoculations:
    - None of the positive controls worked
- Miniprep the liquid inoculations
- Nanodrop:
    - Results varied for both HiFi B inoculations
- RE Digest:
    - Used 40uL nuclease free water, 4uL DNA, 5uL cutsmart, and 1uL EcoRV enzyme
    - Placed into thermocycler at 65C for 20 minutes
- Created 16 plates
- Cleaned the hood with bleach and placed under UV light for 20 minutes

- PCR
    - Used 2x Q5 mastermix protocol for 25 uL reaction
    - For template, a double digested plasmid for Prelim plasmid extension PCR was used
    - For construct plasmid, a PMEI digested plasmid was used
    - Tubes were labeled L-PVT and L-PMY
- Resuspension:
    - Resuspended DNA
    - Created 3 promoter plates and 1 GFP plate
        - Promoters: **BBa_K895000, BBa_K541503, BBa_R0080**
        - GFP: **BBa_E0040**
    - Stored in -20C fridge

---

**Friday July 19th**

- Plates
    - Threw out old plates
    - Placed plates in incubator into the 4C fridge
    - Created and autoclaved KAN and CAP LB broths
- Nanodrop:
    - Nanodropped both PCR constructs (L-PVT and L-PMY)
- Gel Electrophoresis:
    - Added 400uL of TAE and 12uL of Ethidium bromide to fill gel casket
    - For loading, we used 2uL DNA, 3uL water, and 1uL of loading dye
    - Ran for 100V
    - Made two 1% gels
        - 60mL TAE, 1.8uL of Ethidium bromide and 0.6g agarose for each gel
- Opentrons Collaboration
    - Started the human protocol for the fluorescein salt
    - Did Opentrons protocol for fluorescein salt and Abs600
- Organized the 4C fridge
- RE Digest
    - RE digested the big miniprep stock for both prelim and construct
    - Prelim
        - Hind III
        - Sac 1
    - Construct
        - Pme 1
- PCR

    - PCR of main project plasmid and extension PCR of pre lim plasmid
        - RE digest product used as template for PCR
        - Followed protocol for 2X Q5 master mix for 25 uL reaction
    - Used program CPL-58 for main project plasmid
    - Used program EXT-66 for prelim plasmid
    - Left in thermocycler and held at 4C overnight
- Streaking:
    - 3 AMP plates
        - 2 streaked with PVT
        - 1 control streaked with KAN resistant bacteria (PMY)
    - 3 KAN plates
        - 2 streaked with PMY
        - 1 control streaked with AMP resistant bacteria (PVT)
- Liquid inoculation
    - 6 liquid cultures and 1 control
        - 2 EHA 105 in LB
        - 2 GV 3101 in LB + RIF ( 10ug/mL)
        - 2 GV 2260 in LB + RIF ( 10 ug/mL)
    - Placed into the incubator at 28C and 250RPM
- Transformation:
    - Transformated promoter 1,2,3 and GFP into DH5-alpha cells
    - Used 1uL of resuspended DNA from the plates
    - For controls
        - resuspended the DNA from the competency test kit
        - These were positive controls
- Plates:
    - Promoter 1,2,3 on CAM plates
        - Diluted and concentrated
    - GFP on AMP plates
        - Diluted and concentrated
    - Controls
        - Positive
            - R and O on CAM plates
        - Negative
            - R and O on AMP plates
            - GFP on CAM plates

---

**Saturday July 20th**

- Plates:

    - PMY with AMP plates control had growth
    - PVT with KAN plates control had growth
- Made two 70% ethanol bottles
- Gel Electrophoresis
    - Used 400mL TAE and 12uL Ethidium bromide
    - Used a 1% gel
    - Used 3uL water, 2uL DNA, 1uL loading dye
    - Placed 4uL into each well
    - Used 100V to run gel
    - Made another three 1% gels
- Nanodrop - good results
- HiFi:
    - Followed HiFi protocol with 0.5 total pmols of DNA
        - PCR plasmid DNA was used directly
        - Insert DNA was diluted until 0.5uL would need to be added to the reaction
    - Ran reaction for 2 hours
- Transformation:
    - Followed transformation protocol
        - NEB protocol recommended tenfold serial dilutions and plating those results
        - Plates labeled 0.1x were diluted before plating
    - Then placed into an incubator
- Plates:
    - Small growth in negative controls
    - Poor growth on GFP plate
- Liquid inoculation:
    - Promoter 1 (x and y)
    - Promoter 2 (x and y)
    - Promoter 3 (x and y)
    - GFP (x and y)
    - Controls
        - GFP in CAM media
        - Promoter 3 in AMP media

---